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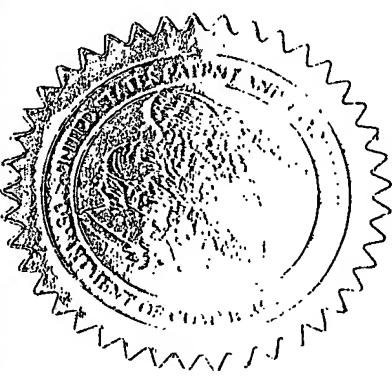
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APPLICATION NUMBER: 60/456,136

FILING DATE: *March 20, 2003*

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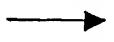
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U.S. PTO
100-998Approved for use through 10/31/2002. OMB 0651-0032
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE**PROVISIONAL APPLICATION FOR PATENT COVER SHEET**

This is a request for filing a PROVISIONAL APPLICATION FOR PATENT under 37 CFR 1.53(c).

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<input type="checkbox"/> Additional inventors are being named on the _____ separately numbered sheets attached hereto		
TITLE OF THE INVENTION (500 characters max) Cyclic Proline-Containing Peptide Mimics		
Direct all correspondence to:		
<input checked="" type="checkbox"/> Customer Number 23910   OR Type Customer Number here		
<input checked="" type="checkbox"/> Firm or Individual Name	Sheldon R. Meyer	
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ENCLOSED APPLICATION PARTS (check all that apply)		
<input checked="" type="checkbox"/> Specification Number of Pages	4	<input type="checkbox"/> CD(s), Number
<input type="checkbox"/> Drawing(s) Number of Sheets		<input type="checkbox"/> Other (specify)
<input type="checkbox"/> Application Data Sheet. See 37 CFR 1.76		
METHOD OF PAYMENT OF FILING FEES FOR THIS PROVISIONAL APPLICATION FOR PATENT		
<input checked="" type="checkbox"/> Applicant claims small entity status. See 37 CFR 1.27.	FILING FEE AMOUNT (\$)	
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The invention was made by an agency of the United States Government or under a contract with an agency of the United States Government.		
<input checked="" type="checkbox"/> No.		
<input type="checkbox"/> Yes, the name of the U.S. Government agency and the Government contract number are: _____		

Respectfully submitted,
SIGNATURE D. Benjamin Borson

Date | March 20, 2003

TYPED or PRINTED NAME D. Benjamin Borson

REGISTRATION NO.
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Docket Number:

TELEPHONE 415.362.3800

42,349
NRNZ-01048US0**USE ONLY FOR FILING A PROVISIONAL APPLICATION FOR PATENT**

This collection of information is required by 37 CFR 1.51. The information is used by the public to file (and by the PTO to process) a provisional application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 8 hours to complete, including gathering, preparing, and submitting the complete provisional application to the PTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, Washington, D.C. 20231. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. SEND TO: Box Provisional Application, Assistant Commissioner for Patents, Washington, D.C. 20231.

Attorney Docket No.: NRNZ-1048US0

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J1040 U.S. PTO
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Synthesis of Cyclic Proline-Containing Peptide Mimics via Ring-Closing Metathesis

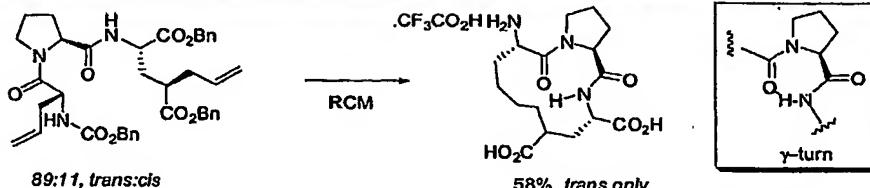
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ABSTRACT



Several dienes embedded in di- and tri-peptides which incorporate proline have been prepared and subjected to ring-closing metathesis. Bicyclic peptide mimics of well-defined amide geometry and of varying ring sizes were prepared. Several limitations of the cyclisation step were revealed.

The use of peptidomimetics¹ to mimic the behavior of biologically active peptides is common in the pursuit of a drug candidate. Peptidomimetics are more metabolically stable and protease resistant than peptides and they often adopt well defined conformations.²

The use of cyclic peptides provides an elegant approach for the synthesis of peptides of rigid geometry that can be used to probe the bioactive conformation of a given peptide. This strategy can be used to establish the structure of a more potent analogue.³

A common method for the synthesis of cyclic peptides⁴ relies on construction of an acyclic precursor, typically a

diene, that then undergoes cyclisation using Grubbs ring-closing metathesis (RCM).⁵ The work reported herein involves the synthesis of dienes from suitable amino acid precursors bearing an allyl group at C(2) or C(5) of proline. RCM of these dienes affords cyclic proline-containing peptides of defined rotamer geometry about the proline amide bond. Several cyclic peptides of varying ring sizes have been prepared and the effect of both stereochemistry and heteroatom location on the metathesis reaction has been investigated.

Synthetic Strategy. We were interested in synthesizing a range of conformationally restricted macrocyclic di- and tri-peptides containing proline, which adopt either *cis* or *trans* stereochemistry about the peptide bond, depending on the substitution on the proline ring.⁶ Given that *cis/trans* isomerase enzymes often deliver the bioactive conformation of a peptide,⁷ peptidomimetics of defined

¹ For recent advances see (a) Aube, J. *Tetrahedron* 2000, **56**, 9725-9842; (b) Gante, J. *Angew. Chem., Int. Ed. Engl.* 1994, **33**, 1699-1720.

² Giannis, A.; Kolter, T. *Angew. Chem., Int. Ed. Engl.* 1993, **32**, 1244-1267.

³ Marshall, G. R. *Tetrahedron*, 1993, **49**, 3547-3558.

⁴ (a) Prabhakaran, E. N.; Rao, I. N.; Boruah, A.; Iqbal, J. *J. Org. Chem.* 2002, **67**, 8247-8250; (b) Banerji, B.; Bhattacharya, M.; Madhu, R. B.; Das, S. K.; Iqbal, J. *Tetrahedron Lett.* 2002, **43**, 6473-6477; (c) Saha, B.; Das, D.; Banerji, B.; Iqbal, J. *Tetrahedron Lett.* 2002, **43**, 6467-6471; (d) Banerji, B.; Malleshwar, B.; Kumar, S. K.; Kunmar, A. C.; Iqbal, J. *Tetrahedron Lett.* 2002, **43**, 6479-6483; (e) Belvisi, L.; Colombo, L.; Colombo, M.; Di Giacomo, M.; Manzoni, L.; Vodopivec, B.; Scolastico, C. *Tetrahedron* 2001, **57**, 6463-6473; (f) Grossmith, C. B.; Senia, F.; Wagner, J. *Synlett* 1999, 1660-1662; (g) Miller, S. J.; Blackwell, H. E.; Grubbs, R. H. *J. Am. Chem. Soc.* 1996, **118**, 9606-9614; (h) Beal, L. M.; Liu, B.; Chu, W.; Moeller, K. D. *Tetrahedron* 2000, **56**, 10113-10125; (i) Hoffmann, T.; Lanig, H.; Waibel, R.; Gmeiner, P. *Angew. Chem., Int. Ed.* 2001, **40**, 3361-3364.

⁵ (a) Grubbs, R. H.; Miller, S. J.; Fu, G. C. *Acc. Chem. Res.* 1995, **28**, 446-452; (b) Schuster, M.; Blechert, S. *Angew. Chem., Int. Ed. Engl.* 1997, **36**, 2037-2056; (c) Armstrong, S. K. *J. Chem. Soc., Perkin Trans. 1* 1998, 371-388; (d) Grubbs, R. H.; Chang, S. B. *Tetrahedron* 1998, **54**, 4413-4450; (e) Fürstner, A. *Angew. Chem., Int. Ed. Engl.* 2000, **39**, 3012-3043; (f) Trnka, T. M.; Grubbs, R. H. *Acc. Chem. Res.* 2001, **34**, 18-29.

⁶ (a) Halab, L.; Lubell, W. D. *J. Peptide Sci.* 2001, **7**, 92-104; (b) Halab, L.; Lubell, W. D. *J. Am. Chem. Soc.* 2002, **124**, 2474-2484.

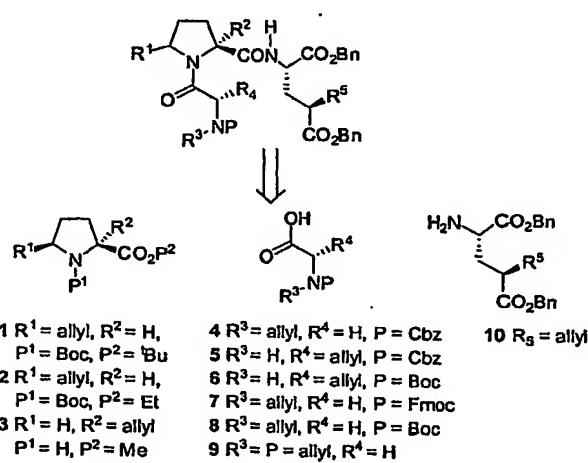
⁷ Fischer, G. *Angew. Chem., Int. Ed. Engl.* 1994, **33**, 1415-1436.

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conformation are useful for incorporation into a peptide scaffold.

In order to prepare a series of *cis* and *trans* bicyclic dipeptides incorporating proline, it was decided to introduce an allyl group at either the C(2) or C(5) position. We chose a glycylprolylglutamic acid scaffold onto which were also placed an allyl group on either the glycine or glutamate residue (Scheme 1). Introduction of an allyl group onto the α -carbon and the nitrogen atom of glycine was deemed straightforward. Glutamic acid was chosen in view of the well-established methodology for the stereoselective introduction of an allyl group at C(4).⁸

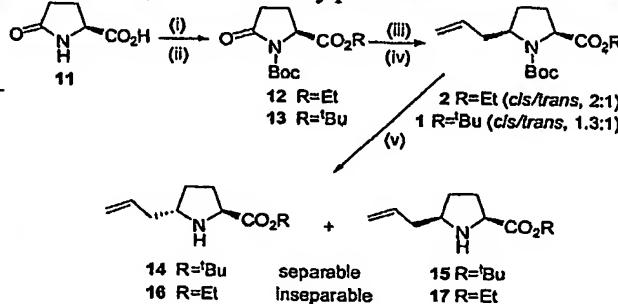
Scheme 1. General synthesis of diene precursors for RCM based on a glycylprolylglutamate scaffold.



Results and Discussion. The synthesis of proline derivatives bearing an allyl group at C(5) has been reported⁹ (Scheme 2). Thus, the ethyl ester¹⁰ and *tert*-butyl ester¹¹ of readily available (*S*)-pyroglutamic acid 11 were protected as their respective Boc carbamates 12¹² and 13.¹³ Selective reduction of the lactam carbonyl with LiEt₃BH yielded aminols, which underwent C-allylation with allyltributylstannane to afford 5-allylprolines 2¹⁴ (*cis/trans*, 66:33) and 1⁹ (*cis/trans*, 57:43) in good yield. Neither set of diastereomers could be separated; however, removal of the Boc group with HCl in dioxane allowed small-scale separation of the *trans* and *cis* *tert*-butyl esters 14 and 15. The stereochemistry of 14 and 15 was determined using nOe experiments and was supported by literature

analogue^{9,10} that suggested that the *cis* isomer 15 would be the major product.

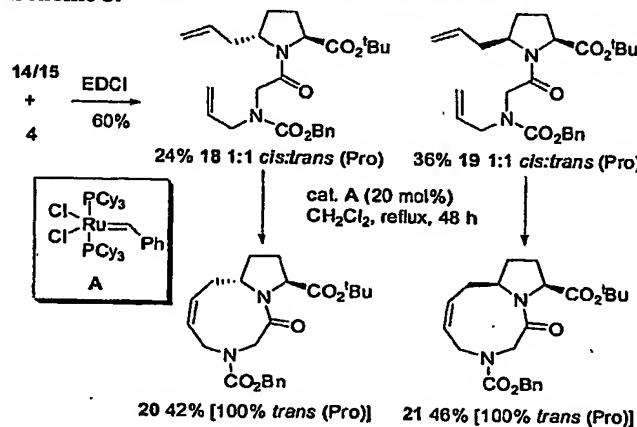
Scheme 2. Synthesis of 5-allylprolines.



(i) for 12, EtOH/SOCl₂, for 13, 70% HClO₄, *tert*-BuOAc (56%); (ii) Boc₂O, DMAP (85% for 2, 95% for 1); (iii) LiEt₃BH; (iv) for 1, allyltributylstannane, Me₂SiOTf (70%) for 2, allyltributylstannane, BF₃.Et₂O (54%); (v) for 1 4 M HCl, dioxane 72% (14 31%, 15 41%); for 2 CF₃CO₂H, ca. 80%.

The preparation of (*S*)-2-allylproline methyl ester 3,¹⁵ *N*-allylglycine carbamates 4,¹⁶ 7,^{17b} 8,¹⁷ of *C*-allylglycines¹⁸ 5¹⁹ and 6,²⁰ and of glutamate 10⁸ followed literature procedures or variations thereof.

Scheme 3.



Coupling of *N*-allylglycine derivative 4 with the mixture of diastereomeric 5-allylprolines 14 and 15 was effected

¹⁵ (a) Seebach, D.; Boes, M.; Naef, R.; Schweizer, W. B. *J. Am. Chem. Soc.* 1983, 105, 5390-5398; (b) Wang, H.; Germanas, J. P. *Synlett* 1999, 33-36.

¹⁶ Hon, Y.-S.; Chang, R.-C. *Heterocycles* 1991, 32, 1089-1099.

¹⁷ (a) Schleich, S.; Helmchen, G. *Eur. J. Org. Chem.* 1999, 2515-2521; (b) Reichwein, J. F.; Liskamp, R. M. J. *Eur. J. Org. Chem.* 2000, 2335-2344.

¹⁸ (a) Bclokon, Y. N.; Taraov, V. I.; Malcev, V. I.; Savel'eva, T. F.; Ryzhov, M. G. *Tetrahedron: Asymmetry* 1998, 9, 4249-4252; (b) Collet, S.; Bauchat, P.; Danion-Bougot, R.; Danion, D. *Tetrahedron: Asymmetry* 1998, 9, 2121-2131.

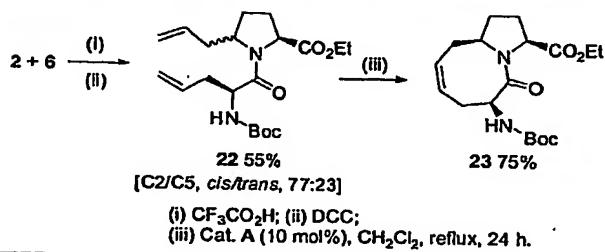
¹⁹ Sun, Z.-Y.; Kwon, C.-H.; Wurpel, J. N. D. *J. Med. Chem.* 1994, 37, 2841-2845.

²⁰ Gao, Y.; Lane-Bell, P.; Vederas, J. C. *J. Org. Chem.* 1998, 63, 2133-2143.

with EDCI to yield the separable dienes 18 and 19 in moderate yield (60%). The *cis/trans* ratio of the newly created amide (Pro) bond in both these flexible acyclic dipeptides was 1:1. Ring-closing metathesis of the individual 1,10-dienes 18 and 19 was accomplished using Grubbs catalyst A to give the expected cyclononenes 20 and 21 in moderate yield (Scheme 3). The ring closure was not affected by the stereochemistry of the allyl group at C(5) on the proline moiety, both reactions giving similar yields with comparable reaction times and catalyst loadings. Very little deallylation/isomerisation occurred (*cf.* *N*- or *O*-allyl systems).²¹ In contrast to their respective precursors, both macrocycles 20 and 21 adopted only the *trans* conformation about the peptide bond.

Subsequent transformations of 20 and 21 proved to be problematic due to the presence of the *tert*-butyl ester and a benzylcarbamate hence our attention was turned to the use of an ethyl ester with a Boc as protecting group. Due to difficulties in separating the ethyl esters 16 and 17, the mixture of diastereomers 2 was coupled with the C(2)-allylglycine 6 using DCC to afford an inseparable mixture of dipeptides 22 [C(2)/C(5), *cis/trans*, 77:23, *cis/trans* (Pro) ratio undetermined due to complex NMR spectra] in 55% yield (Scheme 4). Subjecting the diene mixture to the standard RCM conditions yielded only one macrocycle 23 in excellent yield (75%). Based on the fact that the *cis* diastereomer was the major component in the precursor, the cyclic metathesis product was assigned as *cis* C(2)/C(5) stereochemistry. Clearly the minor *trans* isomer cyclises extremely slowly relative to the *cis* isomer, a trend that was not observed earlier in the metathesis of the *N*-allyl dienes 18 and 19 (Scheme 3). Gratifyingly cyclooctene 23 adopted only *trans* stereochemistry about the peptide bond.

Scheme 4.



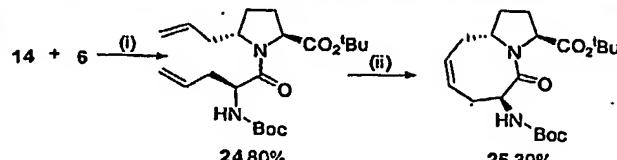
(I) $\text{CF}_3\text{CO}_2\text{H}$; (II) DCC;
(III) Cat. A (10 mol%), CH_2Cl_2 , reflux, 24 h.

In order to access the C2/C5 *trans* diastereomer 25 of 23 it was necessary to use amine 14; coupling with C(2)-allylglycine 6 using DCC yielded the diene 24 in 80% yield [Scheme 5, *cis:trans* (Pro) ratio undetermined due to complex NMR spectra]. As anticipated ring closure of 24 was extremely slow, required high catalyst loading, and afforded a lower yield of the cyclooctene 25. However, the

²¹ (a) Cadot, C.; Dalko, P. I.; Cossy, J. *Tetrahedron Lett.* 2002, 43, 1839-1841; (b) Alcaide, B.; Almendros, P.; Alonso, J. M.; Aly, F. M. *Org. Lett.* 2001, 3, 3781-3784.

peptide bond within macrocycle 25 adopted only a *trans* conformation.

Scheme 5.

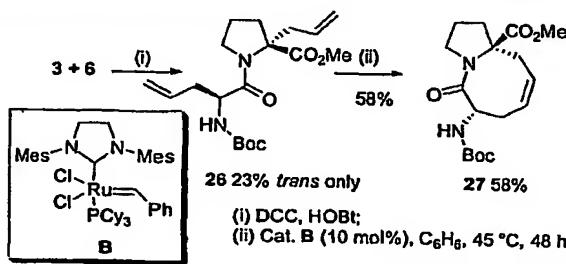


(I) DCC; (II) Cat. A (30 mol%), CH_2Cl_2 , reflux, 116 h.

Grubbs *et al.*²² have reported that certain *cis* substituted cyclohexane derivatives metathesise under more forcing conditions and in lower yield than the corresponding *trans* isomers. In the present work it was observed that placing the allyl moieties closer together (*C*-allyl vs *N*-allyl) and in certain orientations (*cis* vs *trans*) resulted in significant differences in reactivity.

The above metathesis reactions had involved an allyl group at C(5) on the proline and an allyl unit on the glycine, forming macrocycles containing a *trans* (Pro) amide bond. In order to obtain macrocycles with a *cis* (Pro) amide bond, metathesis between an allyl group at C(2) on proline and an allyl group on the glycine was required. Thus metathesis of 26 with the second generation Grubbs's catalyst (B)²³ yielded bicyclic cyclooctene 27th as a single (*cis*) rotamer in good yield (Scheme 6).

Scheme 6.



All attempts to metathesise the corresponding C(2) allyl/*N*-allyl derivatives 28, 29, or 30 (Scheme 7) gave complex mixtures probably due to competing deallylation, which is accelerated by the electron-withdrawing Boc, CO_2Bn or Fmoc carbamates. *N,N'*-Diallylglycine²⁴ derivative 31 was prepared in an attempt to avoid this effect; however, only starting material was recovered after

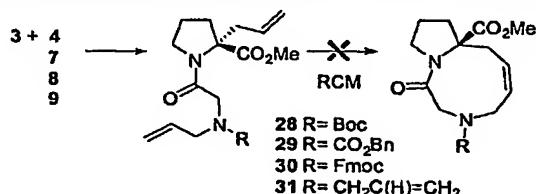
²² Miller, S. J.; Kim, S-H.; Chen, Z-R.; Grubbs, R. H. *J. Am. Chem. Soc.* 1995, 117, 2108-2109.

²³ Morgan, J. P.; Grubbs, R. H. *Org. Lett.* 2000, 2, 3153-3155.

²⁴ (a) Garro-Helion, F.; Merzouk, A.; Guibe, F. *J. Org. Chem.* 1993, 58, 6109-6113; (b) Hassner, A.; Maurya, R.; Friedman, O.; Gottlieb, H. E.; Padwa, A.; Austin, D. *J. Org. Chem.* 1993, 58, 4539-4546.

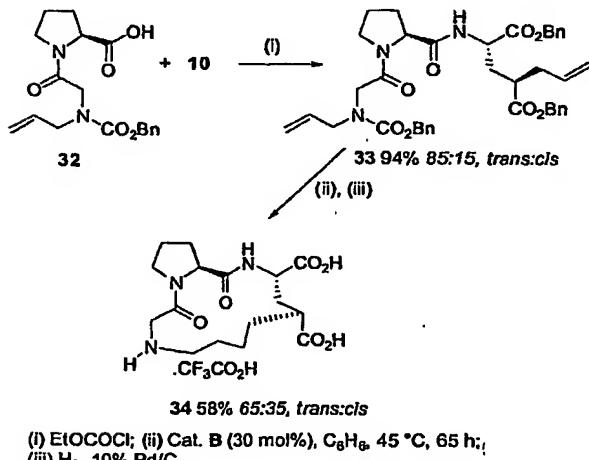
attempted RCM, possibly due to quenching of the catalyst by the reaction with the more basic nitrogen.²⁵ Conversion of diallylamine 31 into its hydrochloride salt²⁶ followed by attempted RCM was also unsuccessful.

Scheme 7.



The ring-closing metathesis of dienes contained within a Gly.Pro.Glu scaffold was also examined. The substrate was synthesized (Scheme 8) from coupling 32 with 10 to yield *N*-allylglycyl diene 33, predominantly as the *trans* (Pro) rotamer. Exposure of 33 to Grubbs catalyst B followed by hydrogenation gave macrocycle 34 in 58% yield after purification by HPLC. Cyclotetradecene 34 existed as a 65:35 mixture of *trans:cis* (Pro) rotamers. The increased proportion of the *cis* rotamer may reflect increased flexibility of the Pro amide bond when it is embedded in a larger 14 membered ring.

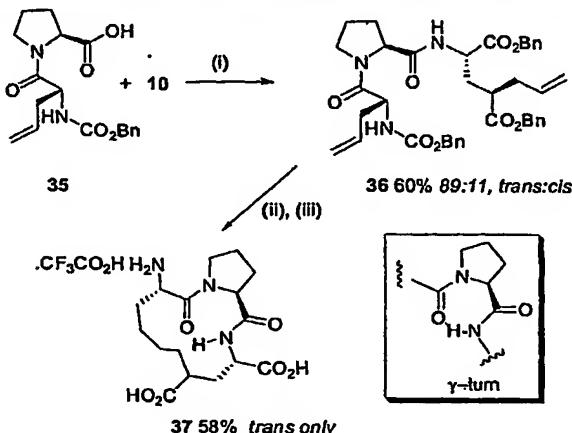
Scheme 8.



Metathesis of the corresponding *C*-allylglycyl dipeptide 36 and subsequent global deprotection yielded the 13-membered macrocycle 37 (58%) as a single *trans* rotamer (Scheme 9). The observation of only the *trans* amide

rotamer may be a consequence of the smaller ring size and/or stabilisation of the structure by the formation of a γ -turn.

Scheme 9.



(i) EIOCOCl; (ii) Cat. A (20 mol%), CH_2Cl_2 , reflux, 48 h;
(iii) H_2 , 10% Pd/C.

Summary. The synthesis and ring closure of a number of di- and tri-peptides incorporating proline has been accomplished via construction of appropriate diallylated peptide precursors and subsequent olefin metathesis. The proline containing peptides cyclised to afford macrocycles that adopted (except for 34) a single conformation about the Gly-Pro amide bond. The conformation adopted in each case however was dependant on both the point of attachment of the diene (*C*-allyl vs *N*-allyl) and on the *cis-trans* stereochemistry [C(2) vs C(5)] on the proline ring.

Acknowledgment. The authors thank NeuronZ Ltd for financial support.

Supporting Information Available: Experimental procedures and spectral data for compounds 20, 23, and 37.

²⁵ Shon, Y-S.; Lee, T. R. *Tetrahedron Lett.* 1997, 38, 1283-1286.

²⁶ Fu, G. C.; Nguyen, S. T.; Grubbs, R. H. *J. Am. Chem. Soc.* 1993, 115, 9856-9857.